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Isolation of Pterocarpanoid Compounds as Heartwood Constituents of *Maackia amurensis* Rupr. et Maxim. var. *Buergeri* Schneid

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Abstract——The isolation and identification of three pterocarpans from the heartwood of *Maackia amurensis* is described on the basis of chemical analysis and spectroscopic means. The pterocarpans identified are 3, 9-dimethoxypterocarpan (homopterocarpin), 3-hydroxy-9-methoxypterocarpan and 3-hydroxy-8, 9-methylenedioxypterocarpan. Occurrence and biogenesis of these pterocarpans are discussed in connection with chemotaxonomy.

Introduction

An investigation of wood extractives has been carried out to obtain information from chemotaxonomical point of view as well as practical aspects such as pitch trouble. In the course of the investigation on wood extractives attempted from the above standpoints, three ptercarpans were isolated from the heartwood of *Maackia amurensis*.

One of them was confirmed to be the same substance as that named Maackiain by Suginome. He reported that maackiain[1] (III), 3-hydroxy-8, 9-methylenedioxypterocarpan**, was isolated from the heartwood of the same material as used in this experiment. However, there have been no reports on isolation of homopterocarpin (I) and 3-hydroxy-9-methoxypterocarpan (IV) in addition to maackiain from the heartwood of *Maackia amurensis*. This report describes the isolation and identification of these pterocarpans.

Maackia amurensis belongs to the family *Leguminosae* (subfamily *Papilionatae*), whose heartwood is characterized by hard and dark brown appearance.

Homopterocarpin and pterocarpin (3-methoxy-8, 9-methylenedioxypterocarpan) (II), which are the known representatives of the pterocarpans, have hitherto been isolated and characterized as heartwood constituents of the pterocarpus species[2,3]. These compounds were first isolated from the heartwood of the red sandal wood tree, *Pterocarpus santalinus*, and their chemical structures were established by Robertson et al.[2] and Späth[4], independently.

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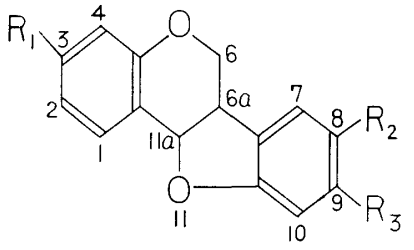
** Nomenclature proposed by Underwood and Harper[8].



I. R_1=OMe, R_2=H, R_3=OMe
II. R_1=OMe, R_2+R_3=·O—CH_2—O·
III. R_1=OH, R_2+R_3=·O—CH_2—O·
IV. R_1=OH, R_2=H, R_3=OMe

Fig. 1.

Stereochemical absolute configuation at 6a and 11a for pterocarpans has been recently elucidated on the basis of analytical data from n.m.r. spectroscopy[5,6].

After then, many pterocarpan derivatives were isolated and their general occurrence in the pterocarpus species, the *papilionatae* and the *caesalipinioideae* of the family *Leguminosae*, has been established[6].

Results and Discussion

Two crystalline compounds were obtained from the *n*-hexane solube fraction of heartwood extract of *Maackia amurensis*. The *n*-hexane soluble fraction was applied to an alumina column and was eluted with the order of *n*-hexane, benzene, ethyl ether and methanol.

One compound obtained from the fraction eluted with ether was identified as β-sitosterol by comparison with an authentic specimen. The other easily crystalizable compound was recovered from the fraction eluted with benzene. The chemical analysis of the compound was found to be consistent with that of homopterocarpin reported in the previous literature. It contained neither hydroxyl nor carbonyl group. ZEIZEL determination indicated the presence of two methoxyl groups. From the result of n.m.r. spectrum, the two sharp absorption signals due to methoxyl proton were observed at τ 6.21 and 6.23 ppm as singlet. The presence of benzene nucleus was confirmed from the results of i.r. spectrum, u.v. absorption spectrum and n.m.r. spectrum.

Phenolic compounds soluble in aqueous sodium hydroxide were obtained from the fraction extracted with cold sodium hydroxide solution of heartwood extract. An attempt was made to separate these phenolic substances by means of silica gel column chromatography with a mixture of chloroform and ethanol (50 : 1 v/v) as an eluting solvent. Portions eluted at the beginning from the column contained pterocarpanoid compounds, and yellow substances were obtained from the latter eluant. Although each of pterocarpanoid compounds and yellow substances were

separated, the former could not be crystalized. Therefore, the compounds were separated by conversion into the acetates, fractional crystallization and hydrolysis of the crystlline acetates with ammonium hydroxide back to original phenol. By means of above procedure, two compounds were separated in crystalline state. One phenolic compound contained a methylenedioxy group and the other contained a methoxy group as well as a phenolic hydroxyl group. The presence of methylenedioxy group was detected by showing positive Labat test. The compound containing a methylenedioxy group was confirmed to be the same substance as maakiain isolated by SUGINOME. Methylation of this compound with diazomethane in ether gave pterocarpin, and the methyl ether was the known compound isolated previouly as a natural product. Methylation of the other compound with diazomethane in ether gave homopterocarpin.

Table 1. N.m.r. spectra of pterocarpans isolated from *Maackia amurensis*
Main assignments of chemical shifts. (τ ppm)

Pterocarpans	1-H	7-H	10-H	11a-H	3-Ac	8,9 $-OCH_2O-$	OMe
3,9-Dimethoxy- (homopterocarpin) (I)	2.50 $J_{1,2}=8.5$	2.87 $J_{7,8}=8.4$		4.50[a]			6.21 6.23
3-methoxy-8,9-methylenedioxy- (pterocarpin)* (II)	2.58 $J_{1,2}=8.5$	singlet 3.27	singlet 3.56	4.52[a]		4.09	
3-Acetoxy-8,9-methylenedioxy- (III)	2.50 $J_{1,2}=8.5$	singlet 3.29	singlet 3.57	4.51[a]	7.73	4.11	
3-Acetoxy-9-methoxy- (IV)	2.47 $J_{1,2}=8.4$	2.88 $J_{7,8}=9.0$		4.50[a]	7.73		6.24

* Data equoted from a literature of Harper and UNDERWOOD et al.[7]
[a] τ value given at the center of doublet.

The structure of the compounds isolated was confirmed by comparison with the result of n.m.r. spectra. The absorption signal due to the proton at C_{11a} splitted into the doublet and showed signs of further long range splitting due to one of the proton at C_6. The n.m.r. spectra of homopterocarpin (I) and the acetate of compound (IV) gave two aromatic absorption bands showing doublet at τ 2.47-2.58 and 2.87-8 ppm, which may be assigned to the proton at C_1 and C_7, respectively[5,7]. An absorption peak at τ 4.11 ppm in the acetate of compound (III) indicates the presence of a methylenedioxy group. In the acetate of compound (IV), on the other hand, an absorption peak due to methoxyl group is observed at τ 6.24 ppm. This result proves that the methoxy group is located at C_9 of pterocarpan nucleus, taking into consideration from the position of two methoxyl groups assigned on homopterocarpin.

The introduction of an acetoxyl group in place of a methoxyl group induces a further deshielding of protons in the meta position by Δ 0.1-0.2[5], and, in con-

formity with this fact, the acetate of compound (IV) has a chemical shift of τ 2.47 ppm toward the side of lower magnetic field.

Recently, HARPER and UNDERWOOD et al.[7] have isolated ten pterocarpanoid constituents from the heartwood of *Swartzia madagascariensis* and determined their chemical structure. They describe the presence of a new pterocarpanoid substituted for hydroxyl or methoxyl group at C_4 of pterocarpan nucleus.

Although pterocarpan group isolated so far from nature includes more than ten compounds, the fact that the compounds are limited to the *papilionatae* and the *caesalipiniodeae* of the family *Leguminosae* is interesting from the point of chemotaxonomy.

3-hydroxy-9-methoxypterocarpan in connection with homopterocarpin was isolated in this experiment. However, pterocarpin as a related substance of maackiain could not be confirmed among pterocarpan group. If a relationship shown in Fig. 2 is assumed, possibly pterocarpin is present in nature in addition to maackiain. Because the heartwood of *maackiain amurensis* is likely to contain maackiain abundantly, pterocarpin may be minute quantitatively.

$$\mathrm{I} \underset{\text{methylation}}{\overset{\text{demethylation}}{\rightleftharpoons}} \mathrm{IV}$$

$$\mathrm{II} \underset{\text{methylation}}{\overset{\text{demethylation}}{\rightleftharpoons}} \mathrm{III}$$

Fig. 2. A relationship assumed structurally among four pterocarpans.

Therefore, it seems most reasonable to consider that maackiain isolated by SUGINOME is not the only charactristic pterocarpanoid constituent of heartwood of *Maackia amurensis*.

Experimental

Preparation of heartwood extractives

Pulverized air dried heartwood (1 kg) was extracted twice with a mixture of benzene and methanol (1 : 1 v/v) for 8 hours. The extracts were concentrated under a diminished pressure to give the deep brown residue (76.4 g). The residue was dissolved in a small amount of methanol and extracted by shaking with *n*-hexane three times in order to remove *n*-hexane soluble fraction. The combined *n*-hexane extracts were evaporated to pale brown gums (3.8 g) (yield 0.4 % based on air dried heartwood powder). After removal of the *n*-hexane soluble fraction, the residue was extracted by shaking with ice cold 5 % sodium hydroxide solution, and then the insoluble fraction was filtered off. The sodium hydroxide soluble

fraction was neutralized rapidly with ice cold dilute hydrochloric acid and extracted with ether. The ether extract was dried over anhydrous sodium sulfate, filtered and evaporated to almost dryness (26 g) (yield 2.8 %).

Isolation of two crystalline compounds from n-hexane soluble fraction

The fraction extracted with *n*-hexane was applied to a column packed with a suitable amount of alumina for chromatography, and eluted with *n*-hexane, ethyl ether and methanol, successively. Each fraction was collected separately, and examined by using thin layer plates of silica gel H. The following mixture was used as the developing solvent; (A) benzene and petroleum ether (4 : 1 v/v), (B) benzene and ethyl acetate (9 : 1 v/v), and the compounds on the thin layer plate was detected by spraying with concentrated sulfuric acid.

The fraction eluted with *n*-hexane gave an oily mixture giving three spots with Rf values of 0.68, 0.76, 0.95 with solvent (A), but further identification procedures were not carried out.

The fraction eluted with benzene was composed of two main components, whose Rf values were 0.43 and 0.80 with solvent (B). The former spot showed blue color and the latter did orange color. The Rf value of the former spot corresponded with that of an authentic β-sitosterol, and a similar compound was also obtained from the fraction eluted with ethyl ether. The fraction eluted with methanol consisted of various components which could not be crystallized, and so further investigation was not carried out. Rechromatography with silica gel column was performed in order to separate two main compounds in the fraction eluted with benzene. Each of them could be separated in crystalline state, and recrystallized with methanol or cold benzene. One of the crystalline compound separated gave a typical sterol color reaction changing to blue and then green with Liebermann-Burchard test.

Identification of β-sitosterol

M.p. and mixed m.p. 137°C (recryst. from MeOH) lit. 137°C
$[\alpha]_D$ −35.5° (c, 2.0 $CHCl_3$) lit. −37° ($CHCl_3$)
Anal. Found: C, 82.24; H, 12.02;
Calcd. for $C_{29}H_{10}O$ 1/2 H_2O: C, 82.20; H, 12.05 %
Acetate m.p. and mixed m.p. 128°C lit. 128°C

The other crystlline compound which gave single spot on thin layer chromatogram was obtained as needles. The compound was identified as homopterocarpin on the basis of the result of chemical analyses.

M.p. 88–9°C lit. 87–8°C, 83–4°C, $[\alpha]_D$ −205° (c, 1.0 $CHCl_3$) lit. −225°, −207°
ν 1620, 1495 cm^{-1} (aryl), λ_{Max}^{EtOH} 286 nm (log ε 3.98)

Anal. Found: C, 71.63; H, 5.60; OMe 20.6 %

Calcd. for $C_{15}H_{10}O$ $(OMe)_2$ (M.W. 284 from Mass spectrum): C, 71.83; H, 5.63% OMe 21.8 %

Isolation of pterocarpanoid compounds

The sodium hydroxide soluble portion was placed on a silica gel column, and was eluted with a mixture of chloroform and ethanol (50 : 1 v/v). The eluate from the column was collected with a fraction collector, and examined by means of thin layer chromatographic analysis. Fraction (I) showing Rf value 0.4-0.6 and fraction (II) of Rf value 0.1-0.3 with the developing solvent (B) were separated roughly. Fraction (I) contained pterocarpanoid compounds giving a red color or an orange color with concentrated sulphuric acid. From fraction (II) collected and evaporated, yellow substances were recovered as powder by means of preparative thin layer chromatography with a mixture of chloroform and methanol (9 : 1 v/v).

Although this substance gave a yellow spot having Rf value 0.58-9 on thin layer chromatogram with the above developing solvent, it did not give a distinctive melting point.

On spraying with concentrated sulphuric acid on the thin layer chromatogram, yellow coloration of the spot increased and another new yellow spot at Rf 0.60 appeared overlapped. This spot indicated fluorecence with u.v. light. Thus, the yellow powder obtained was found to be a mixture composed of two compounds. Further investigations on this yellow substance are in progress. Fraction (I) collected and evaporated was dissolved in ethyl acetate saturated with water, and the solution was subjected to the alumina column and eluted with ethyl acetate containing water. The portions containing the pterocarpanoid compounds were collected and evaporated to a viscous syrup. Pterocarpanoids with phenolic hydroxyl group failed to be crystalized and so were converted into their acetates with pyridine and acetic anhydride at room temperature. The crystalline acetates obtained by recrystalization with ethyl acetate were found to consist of at least two compounds. The mixture of acetates was treated with cold ethyl acetate, and an insoluble component was crystllized from ethyl acetate repeatedly to give 3-acetoxy-8, 9-methylenedioxypterocarpan as needles.

M.p. 174-5°C lit.[7] 176-7°C

$[\alpha]_D$ −180° (c, 1.0 $CHCl_3$) lit.[7] −179° ($CHCl_3$), −176° ($CHCl_3$)

ν^{KBr} 1755 (C=O) 1615, 1590, 1500 cm^{-1} (aryl)

λ_{Max}^{EtOH} 284, 309 nm (log ε 3.67, 3.81)

Anal. Found: C, 66.80; H, 4.40 Ace. 13.9 %

Calcd. for $C_{16}H_{11}O_4$ (OAc) (M.W. 326 from Mass spectrum): C, 66.26; H, 4.29 % Ace. 13.2 %

The filtrate of the solution of cold ethyl acetate was reduced to dryness, and the residue was crystallized repeatedly from ethyl acetate and petroleum ether to give 3-acetoxy-9-methoxypterocarpan as needles.

M.p. 120–1°C lit.[8] 122–3°C, $[\alpha]_D$ −181° (c, 1.15 $CHCl_3$)
ν^{KBr} 1755 (C=O) 1620, 1590, 1500 cm^{-1} (aryl)
λ_{Max}^{EtOH} 285, 310 nm (log ε 3.86, 3.19)
Anal. Found: C, 68.73; H, 4.97 OMe 9.6 %
Calcd. for $C_{17}H_{13}O_4$ (OMe) (M.W. 312 from Mass spectrum): C, 69.2; H, 5.2 % OMe 9.9 %

The acetate was dissolved in ethanol and refluxed with 28 % ammonium hydroxide solution for 10 min. The product was isolated by dilution of the reaction mixture with water and subsequent extraction with ether. Ether was evaporated and the residue was crystallized from ethanol to give 3-hydroxy-9-methoxypterocarpan.

M.p. 125–6°C lit.[8] 127.5–8.5°C, $[\alpha]_D$ −234° (c, 1.0 $CHCl_3$) lit.[8] −226° ($CHCl_3$)
λ_{Max}^{EtOH} 286, 281 nm (log ε 3.66, 3.61)
Anal. Found: C, 70.61; H, 5.09 OMe 10.2 %
Calcd. for $C_{15}H_{11}O_3$ (OMe): C, 71.1; H, 5.2 % OMe 11.5 %

3-hydroxy-9-methoxypterocarpan was methylated with diazomethane in ether, and it was confirmed to be corresponded with homopterocarpin by thin layer chromatography.

Literature

1) SUGINOME, H., Experimentia, **15**, 161 (1962).
2) MCGOOKIN, A., ROBERTSON, A. and WHALLEY, W. B., J. Chem. Soc., 787 (1940).
3) AKISANYA, A., BEVAN, C. W. L. and HIRST, J., J. Chem. Soc., 2679 (1959).
4) SPÄTH, E. and SCHLAGER, J., Ber. dtsch. Chem. Ges., **73**, 1 (1940).
5) PACHLER, K. G. R. and UNDERWOOD, W. G. E., Tetrahedron, **23**, 1817 (1967).
6) MABRY, T. J., ALSTON, R. E. and RNUECKLES, V. C., Recent Advances in Phytochemistry, Vol. **1**, North-Holland Publishing co., New York, 1968, p. 350.
7) HARPER, S. H., KEMP, A. D., UNDERWOOD, W. G. E. and (in part) CAMPBELL, R. V. M., J. Chem. Soc., (C) 1109 (1969).
8) HARPER, S. H., KEMP, A. D. and UNDERWOOD W. G. E., Chem. Ind., **27**, 562 (1965).